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THE TEMPERATURE OF PASTEURIZATION FOR BUTTER MAKING.

BY

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CONTENTS.

ILLUSTRATIONS.

THE TEMPERATURE OF PASTEURIZATION FOR BUTTER MAKING.

By L. A. Rogers, *Bacteriologist;* W. N. Berg, *Chemist;* and Brooke J. Davis, *Assistant, Dairy Division.*

INTRODUCTION.

The pasteurization of cream for butter making has for its primary object the elimination of the normal bacteria of the cream to enable the butter maker by controlling the ripening of the cream to secure a uniform product. Incidentally, it may remove some of the possible causes of the deterioration of the butter, as well as destroy the pathogenic bacteria and expel some of the gases and other volatile flavor-giving substances.

There is no fixed standard for the temperature of pasteurization in this country. In Denmark, where all cream used in butter making is pasteurized, a temperature of 82° to 85° C. (180° to 185° F.) is used, but in this country the cream is frequently heated to not more than 63° C. (145° F.), and rarely above 77° C. (170° F.).

It is obvious that it is desirable to determine the most effective temperature at which cream should be pasteurized for butter making. This temperature is the one at which the greatest number of undesirable factors are eliminated with the minimum effect on the cream itself. Several factors are involved in the determination of this temperature, among the most important of which are the uniform destruction of a large proportion of the bacteria of the cream; the destruction of the enzyms inherent in the milk; the avoidance of imparting scorched, metallic, or other undesirable flavors to the cream; and the possible increased loss of fat in the buttermilk.

This paper gives the results of an investigation which had for its object the determination of the proper temperature for the pasteurization of cream for butter making as indicated by the destruction of the bacteria and the enzyms and by the changes in flavor of the butter in storage.

METHOD OF HANDLING CREAM IN EXPERIMENTAL WORK.

The cream used came partly from milk separated at the creamery with which the field laboratory is connected and partly from hand separators. It was all sweet and of fair quality. The pasteurization was done in a continuous Jensen machine. The temperature was

controlled by a hand valve and determined by a naked chemical thermometer inserted in the cream pipe near the machine. The variation was not over 1½° F., plus or minus. The cream was cooled at once to churning temperature and churned within three hours. This method was followed to avoid the complications due to poor starters, to contamination during ripening, or other factors which might change the flavor of the butter and obscure the influence of the pasteurization temperature. These results, therefore, throw no light on the effect of the growth of bacteria during the ripening of imperfectly pasteurized cream. Obviously, also, it does not necessarily follow that similar results would be obtained from cream which was sour or otherwise fermented at the time of pasteurization.

BACTERIOLOGICAL RESULTS OF PASTEURIZING CREAM.

The results for the first season's work are given in Table 1 and those obtained in the second season in Table 2. Bacteria were determined in composite samples of the pasteurized cream the first season, and in the second season the count of the raw cream was taken also. Lactose litmus gelatin was usually employed for plating, but in some cases lactose agar was used. The gelatin plates were incubated at 20° to 22° C. (68° to 72° F.) for five days and the agar plates at 30° C. (86° F.) for three days.

The lack of any established standard of bacteriological efficiency makes it difficult to draw conclusions from the bacteriological results alone. The number of bacteria remaining in the cream can not be taken as an absolute standard of the efficiency of the pasteurization, since this number varies not only with the temperature and time of pasteurization but also with the number and kinds of bacteria present in the cream before pasteurization. The percentage reduction is equally unreliable, as this also is largely dependent on the number of bacteria in the cream before pasteurization. A large number of bacteria in pasteurized cream may simply mean that the original cream contained a very great number of bacteria, or that a large proportion were heat resistant. If the number in the cream before pasteurization was very high, a large number may be left in the cream after pasteurization and the result still show a high percentage reduction. This is illustrated by the results obtained for sample 70 in Table 2, in which with nearly 1,000,000 bacteria remaining in the cream after pasteurization a reduction of 99.5 per cent was obtained. The influence of the kind of bacteria occurring in the cream is seen in a comparison of samples 54 and 70, both pasteurized at 82° C. (180° F.). In sample 54, 205,500,000 bacteria were reduced to 14,000, a reduction of 99.9 per cent, while with sample 70, 172,000,000 were reduced to 940,000, giving a reduction of 99.5 per cent. A large proportion of

the bacteria in sample 54 were evidently in the vegetative stage and easily destroyed, while sample 70 contained many resistant spores.

The cream used in the first season's work was of good quality and about half of the results were obtained in September when the bacterial content was low. In the second season the proportion of hand-separator cream was greater and its inferior quality was indicated by the higher number of bacteria in the pasteurized cream. In Table 1 fairly uniform results are shown at 71° C. (160° F.) and above, and it is evident that 66° C. (150° F.) is too low to secure efficient pasteurization. The results given in Table 2 show efficient pasteurization at 77° C. (170° F.) and higher, and only fair results at 71° C. (160° F.).

It is evident from these results that with cream of good quality efficient pasteurization from the bacteriological standpoint can be secured by momentary heating to 71° C. (160° F.). This is, however, near the lower limit of safety, and if the bacterial content of the raw cream is high a temperature of 74° to 77° C. (165° to 170° F.) must be used to secure uniform results.

TABLE 1.—*Bacteria in cream after pasteurization—First season.*

No. of sample.	Pasteurizing temperature.		Bacteria per cubic centimeter.	No. of sample.	Pasteurizing temperature.		Bacteria per cubic centimeter.
	°C.	*°F.*	*Number.*		*°C.*	*°F.*	*Number.*
1	66	150	1,525,000	38	74	165	61,000
2	66	150	960,000	41	74	165	15,000
3	66	150	1,695,000	14	77	170	46,500
4	66	150	1,630,000	16	77	170	38,000
44	66	150	54,000	18	77	170	78,500
5	68	155	686,300	20	77	170	145,000
7	68	155	331,500	22	77	170	34,650
9	68	155	1,099,000	36	77	170	23,300
11	68	155	406,000	39	77	170	17,600
42	68	155	43,000	23	79	175	6,050
45	68	155	13,500	25	79	175	12,800
6	71	160	395,500	27	79	175	7,900
8	71	160	686,500	29	79	175	8,100
10	71	160	162,000	37	79	175	24,600
12	71	160	189,500	24	82	180	29,800
40	71	160	16,500	26	82	180	14,900
43	71	160	26,000	28	82	180	4,550
13	74	165	50,500	30	82	180	33,350
15	74	165	18,500	35	82	180	17,000
17	74	165	191,500	31	88	190	124,000
19	74	165	244,000	33	88	190	4,500
21	74	165	80,500				

AVERAGES OF TABLE 1.

Number of tests.	Pasteurizing temperature.		Bacteria per cubic centimeter.	Number of tests.	Pasteurizing temperature.		Bacteria per cubic centimeter.
	°C.	*°F.*	*Number.*		*°C.*	*°F.*	*Number.*
5	66	150	1,172,800	7	77	170	54,780
5	68	155	449,560	5	79	175	11,910
6	71	160	246,000	5	82	180	16,880
7	74	165	95,800	2	88	190	64,250

TABLE 2.—*Bacteria in cream before and after pasteurization—Second season.*

No. of sample.	Pasteurizing temperature.		Per cubic centimeter in raw cream.	Bacteria per cubic centimeter in pasteurized cream.	Per cent reduction.	No. of sample.	Pasteurizing temperature.		Per cubic centimeter in raw cream.	Bacteria per cubic centimeter in pasteurized cream.	Per cent reduction.
	°C.	*°F.*	*Number.*	*Number.*	*Per cent.*		*°C.*	*°F.*	*Number.*	*Number.*	*Per cent.*
46	......	......	6,900,000	....	....	66	71	160	....	1,950,000	....
60	......	......	92,000,000	....	....	56	77	170	45,900,000	160,000	99.7
47	60	140	6,900,000	74,500	98.9	67	77	170		56,500	....
48	60	140	4,470,000	680,000	84.8	68	77	170	122,000,000	11,000	99.9
50	60	140	230,000,000	2,950,000	98.7	53	80	176	180,000,000	64,000	99.9
61	60	140	92,000,000	7,250,000	92.1	54	82	180	205,500,000	14,000	99.9
62	60	140	86,500,000	18,400,000	78.7	69	82	180	122,000,000	12,500	99.9
49	66	150	4,470,000	740,000	83.4	70	82	180	172,500,000	940,000	99.5
58	66	150	136,600,000	775,000	99.4	55	88	190	205,500,000	24,500	99.9
63	66	150	86,500,000	1,155,000	98.7	57	88	190	45,900,000	324,600	99.3
64	66	150	38,000,000	795,000	97.9	71	88	190	172,500,000	64,000	99.9
51	71	160	230,000,000	1,000,000	99.6	72	88	190	19,300,000	9,500	99.9
52	71	160	180,000,000	100,000	99.9	59	93	200	136,600,000	23,500	99.9
65	71	160	38,000,000	304,500	99.2	73	93	200	19,300,000	18,000	99.9
AVERAGES OF TABLE 2.											
5	60	140	83,774,000	5,870,900	93.0	3	82	180	166,500,000	322,130	99.8
4	66	150	66,142,500	866,750	98.7	4	88	190	110,825,000	105,525	99.9
4	71	160	149,333,000	838,600	99.4	2	93	200	77,950,000	20,750	99.9
3	77	170	83,950,000	75,830	99.9						

TESTS FOR THE PRESENCE OF ENZYMS.

Certain enzyms occur normally in milk. When cream is churned these enzyms pass over into the butter. Their action is as yet undetermined, but it is possible that they take some part in the changes which occur in butter, even at the low temperatures of commercial storage. This possibility makes it desirable that they be destroyed in cream used for making butter.

The following definitions of the milk enzyms are taken for granted in this paper, no attempt being made to be rigorously exact from the biochemical point of view:

Peroxidase: An enzym that oxidizes other substances by transferring oxygen to them from some peroxid, such as hydrogen peroxid.

Catalase: An enzym that decomposes hydrogen peroxid, forming water and oxygen.

Galactase: The proteolytic enzym of milk.

Lipase: An enzym that splits fats (or fatty esters) into free fatty acids and the corresponding alcohols.

Although normal milk (or cream) probably contains no oxidase, this enzym was always looked for when testing for peroxidase. Up to the present time it has not been found in the materials tested. The naturally occurring oxidases are looked upon as mixtures of a peroxidase and a peroxid, or as mixtures of a peroxidase and a substance that can easily form peroxids.

Enzyms are unstable substances or agents and are easily destroyed by high heat. For convenience, the temperature at which an enzym

is destroyed is called its thermal death point, although an enzym being unorganized can not have a real death point. It should be remembered, however, that the temperature at which any particular enzym can be destroyed is somewhat indefinite, the enzym being gradually weakened by heat when the temperature approaches the death point or when the time of exposure is lengthened. Furthermore, the exact temperature at which it is destroyed varies with the conditions under which it is exposed to the heat. The reaction of the medium, the presence or absence of the substrate, the amount of moisture present, etc., may raise or lower the thermal death point.

During the summer of 1908 tests for peroxidase, catalase, and galactase were made on samples of cream. Formaldehyde solution (40 per cent) was added to the samples—1 or 2 liters of cream—in the proportion of 1 to 1,000. After the addition of the preservative portions of the sample were withdrawn for use and the remainder stored at room temperature. This method of preservation was found to be ineffective, since it did not prevent the growth of mold in many of the samples. Furthermore, the cream would not remain uniform in its composition in spite of repeated attempts to redistribute the fat by mixing. On account of the large amount of fat present chloroform in ordinary amounts could not be used. Although proper sampling at the end of the summer was impossible, the material was used to obtain approximate results for future guidance. In this paper are incorporated only those results of the tests for peroxidase and catalase which were made in the beginning of the summer. By suitable controls it was easily ascertained that the results of the tests were not appreciably affected by the preservative used.

During the summer of 1909 the tests for peroxidase and catalase were made on samples of cream obtained from the creamery soon after pasteurization.

For the work on galactase the buttermilk obtained after the churning of the different lots of cream was used. The samples of buttermilk were preserved at room temperature in stoppered bottles, 5 c. c. of chloroform being added to 1 liter of buttermilk. This concentration of chloroform has been found to have very little effect on the proteolytic enzym of milk.[1] Suitable bacterial counts showed that it preserved the buttermilk very efficiently. Toward the end of the summer tests for peroxidase and catalase were made on the samples of buttermilk to confirm the tests previously made on cream and to ascertain whether these enzyms were affected or destroyed during the changes that had meanwhile taken place. It was apparent from

[1] Harding, H. A., and Van Slyke, L. L. Chloroform as an aid in the study of milk enzyms. New York Agricultural Experiment Station, Bulletin No. 6. Geneva, 1907.

the qualitative tests for these enzyms that they were not yet very strongly affected (see pp. 313, 315).

The results for lipase were obtained in the early part of 1910 in the laboratories at Washington. Portions of the same sample of milk were pasteurized in a small apparatus specially constructed for the purpose, and the effect of the pasteurization on the activity of the lipase present was determined.

PEROXIDASE.

The method of testing for peroxidase was as follows: A small amount—5 to 10 c. c.—of cream (milk or buttermilk or curd solution) was transferred to a test tube. Two or three drops of a freshly prepared alcoholic solution (about 10 per cent) of gum guaiac were allowed to run down the side of the test tube so that the tincture remained on the upper surface of the liquid to be tested. The tube was allowed to stand 5 to 10 minutes. The nonappearance of color indicated the absence of oxidase. Two or three drops of a dilute solution of hydrogen peroxid (or old turpentine) were added, and if peroxidase was present a blue color developed where the reagents came in contact. Usually a blue ring on or near the upper surface of the liquid was formed.

Suitable controls were made to insure that the results would not be vitiated by variations in the degree of acidity of the cream, by the amount of preservatives used, or other factors. The peroxidase and catalase reactions were as strong in very sour raw cream or milk as in the original samples.

Results for peroxidase.—In Table 3 are summarized the results of the tests on the various samples of cream. Tests made on the corresponding samples of buttermilk when three months old gave practically identical results. From the table it is obvious that when cream was pasteurized under the conditions described on page 307 the peroxidase reaction was not obtained in cream pasteurized at 79° C. (175° F.) or above; at 77° C. (170° F.) it was generally absent, and in cream pasteurized at 74° C. (165° F.) or below positive tests were always obtained.

TABLE 3.—*Showing destruction of peroxidase and catalase in cream by pasteurization.*

Temperature of pasteurization.		Test for peroxidase.	Test for catalase.
°C.	*°F.*		
Raw.	*Raw.*	+	+
60	140	+	+
66	150	+	Weak.
68	155	+	....
71	160	+	Very weak.
74	165	+	—
77	170	±	—
79	175	—	—
82	180	—	—
88	190	—	—
93	200	—	—

In general, these results are in accord with those obtained by other investigators who pasteurized for comparatively short periods of time. It must be borne in mind, however, that the death temperature of an enzym is so strongly influenced by so many conditions that an exact agreement between the above results and those obtained by other investigators can hardly be expected. Thus, Herholz [1] found that milk heated one minute at 75° C. (167° F.) gave an uncertain test with guaiac for peroxidase. Hippius [2] obtained the same result with milk heated in exactly the same way. Wilkinson and Peters [3] found that at 78° C. (172° F.) the guaiac test for peroxidase became negative. They do not say how long the milk was maintained at that temperature.

The question of how long the peroxidase can exist in butter under ordinary conditions of storage is important, since the peroxidase present in butter, in spite of the absence of a peroxid, may be able to transfer slowly some of the inclosed oxygen to the several oxidizable substances present. It is possible that such slow oxidation may be materially assisted by the presence of organic salts of iron or of other metals and by the fine subdivision of the inclosed oxygen by overworking the butter.[4]

The tests for peroxidase in buttermilk were repeated in June, 1910, when the samples were one year old. The reaction was given by only about half the samples that had previously given it, clearly indicating the instability of the peroxidase. The disappearance was probably brought about by the slow digestion that had taken place. The peroxidase reaction in raw milk can be made to disappear in about 20 or 25 days, by allowing the milk to sour, adding chloroform to eliminate subsequent bacterial action, and then allowing proteolysis by the lactic acid to go on, either with or without the aid of pepsin.

Peroxidase in raw-cream butter can be detected as follows: Melt the sample at about 45° C. (113° F.), let the curd solution settle to the bottom of the beaker, pour off the clear supernatant fat, and use 5 to 10 c. c. of the curd solution for the test with guaiac, as usual. All the samples thus tested (five, fresh) gave positive reactions, although the reaction appeared to be weak when compared with the same reaction in raw cream.

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[1] Herholz. Beiträge zu bisher bekannten Reaktionen zur Unterscheidung von roher und erhitzter Milch mit besonderer Berücksichtigung der Guajakproben. Dissertation. P. 70, Table C. Braunsberg, 1908.

[2] Hippius, Alexander. Biologisches zur Milchpasteurisierung. Jahrbuch für Kinderheilkunde, Band 61, p. 375. Berlin, 1905.

[3] Wilkinson, W. Percy, and Peters, Ernst R. C. Eine neue Reaktion zur Unterscheidung von roher und erhitzter Milch sowie zum Nachweise von Wasserstoffsuperoxyd in der Milch. Zeitschrift für Untersuchungen der Nahrungs- und Genussmittel. Band 16, Heft 3, pp. 172–174. Berlin, 1908.

[4] Rogers, Lore A. Fishy flavor in butter. U. S. Department of Agriculture, Bureau of Animal Industry, Circular 146, p. 12. Washington, 1909.

Five samples of butter that had been in cold storage for periods ranging from 1½ to 4½ years were tested for peroxidase. They were contained in ordinary tin cans, and only the inner portions of the samples were used because of the presence of iron rust on that part of the sample in contact with the can. All of these gave such unusually strong peroxidase reactions with tincture of guaiac and hydrogen peroxid as to suggest an unusual cause. In marked contrast to raw cream, these curd solutions obtained from old butter when boiled and cooled still gave the peroxidase reaction. Even repeated boiling and cooling did not remove from this material the property of giving this reaction. Evidently the peroxidase in raw cream is different from that present in these samples of old butter.

In view of the fact that the catalytic oxidation of guaiac resin and of other substances by metals or their salts has frequently been observed,[1] it was natural to suppose that the peroxidase reaction in the boiled curd solutions was due not to the enzym occurring normally in raw milk, but to some metallic compound of extraneous origin. The old-curd solutions were qualitatively tested for iron by adding to the unconcentrated material a few drops of dilute hydrochloric acid and potassium ferrocyanid or ammonium sulphocyanate. Positive tests were easily obtained. Control tests showed that much more iron was present in the curd solutions than in milk. Raw milk or buttermilk containing approximately 0.1 per cent of iron added as chlorid or sulphate will give the peroxidase reaction even after being boiled. It would seem reasonable to attribute the peroxidase reaction in the boiled curd solutions to their iron content.

Observations such as these may be of value in explaining the detrimental influence of rusty milk cans, etc., on the flavor and keeping quality of butter.[2]

CATALASE.

The method of testing for catalase was as follows: Fifty cubic centimeters of cream, milk, or buttermilk was introduced into a 100 c. c. Erlenmeyer flask and 25 c. c. of commercial hydrogen peroxid solution added. The flask was quickly closed with a rubber stopper provided with a bent glass tube, the other end of which was inserted into a fermentation tube (capacity 20 c. c.) filled with water. If raw cream were used, the oxygen liberated by the catalase rose in the fermentation tube and completely filled it three or four times in a few minutes. With raw milk or pasteurized cream the liberation

[1] Alsberg, Carl A. Beiträge zur Kenntnis der Guajak-Reaktion. Archiv für Experimentelle Pathologie und Pharmakologie. Supplementband 1908. Festschrift Prof. Oswald Schmiedeberg, pp. 39–53. Leipzig, 1908.

Colwell, Hector C. The catalytic oxidation of guaiac resin by metallic copper. Journal of Physiology, vol. 39, pp. 358–360. London, 1909–1910.

[2] Olson, George A. Rusty cans and their effect upon milk for cheesemaking. Wisconsin Agricultural Experiment Station, Bulletin 162. Madison, 1908.

of gas was slower, until with cream pasteurized at high temperature only a few bubbles were obtained even on long standing. By this method the distinction between raw cream and that in which catalase has been destroyed was easy to make. The above proportions of cream and hydrogen peroxid were used because they were found to be convenient; slight deviations from them do not detrimentally affect the results.

Results for catalase.—From the results (obtained in June, 1909) summarized in Table 3, it is apparent that the catalase in cream was destroyed when pasteurized at 70° to 71° C. (158° to 160° F.). This is a few degrees below the temperature at which peroxidase is destroyed. Confirmatory tests made in September, 1909, on the corresponding samples of buttermilk gave similar results. The tests on buttermilk were repeated in June, 1910. None of the samples then gave the catalase reaction except No. 59, from cream pasteurized at 93° C. (200° F.), which gave it strongly. Accidental bacterial contamination was the probable cause. It did not give the peroxidase reaction, showing that in this instance, at least, the two reactions are due to two different substances or agents. This has been observed before.[1]

Catalase in appreciable amounts probably is not present in butter made from properly pasteurized cream. As a possible factor influencing the quality of storage butter it may obviously be left out of consideration when butter is made from pasteurized cream. A discussion of its possible action in raw-cream butter must be deferred until more data are obtained.

GALACTASE.

METHOD OF MEASURING THE ACTIVITY OF GALACTASE IN BUTTERMILK.

To determine whether the galactase in cream was destroyed or partly inactivated at the different pasteurizing temperatures, water-soluble nitrogen was determined in the buttermilk shortly after churning and after preservation (with chloroform) for an average of 83 days at room temperature.

Into a 500 c. c. volumetric flask 50 to 200 c. c. of buttermilk was introduced. The same volume of buttermilk was used at the beginning and at the end of the period. Distilled water was added up to about 400 to 450 c. c. One-fifth normal acetic acid was slowly and carefully added until the casein separated completely in large flocculi, leaving the supernatant liquid practically water clear. For amounts of acetic acid used see Table 5. Distilled water was added to the 500 c. c. mark, and the mixture was filtered on a 32 cm. folded filter (S. & S. No. 588 or 595) into a clean, dry 500 c. c. volumetric

[1] Kastle, J. H. The oxidases and other oxygen-catalysts concerned in biological oxidations. U. S. Treasury Department, Public Health and Marine-Hospital Service, Hygienic Laboratory, Bulletin 59. Washington, 1910.

flask. During the filtration the funnel was covered with a well-fitting watch glass to minimize evaporation. The first part of the filtrate was returned to the filter two or three times until the filtrate was freed from all particles of suspended protein. When 200 c. c. of buttermilk were used the filtrate was colored faintly yellow and was not more opalescent than other solutions of equal protein content. In two 200 c. c. portions of the filtrate total nitrogen was determined by the usual Kjeldahl method. The remaining part of the filtrate was measured and the total amount recovered noted. The figures were recorded to make certain that the amount of filtrate recovered did not vary enough to affect the results appreciably. Variations in the amount of filtrate evaporated will introduce little or no error into the calculations, if the same amounts of buttermilk are used at the beginning and the end of the period. The average amount of filtrate recovered was 465 c. c.; both in June and in September, 1909, it varied between 445 and 490 c. c. Some of the filtrate was of course retained by the precipitate on the filter paper.

With a little practice the amount of acetic acid to be used in effecting the complete precipitation of the casein can be accurately determined by noting the appearance of the precipitate and of the liquid in which it is suspended. The precipitation is very nearly maximal when the casein separates out in large flocculi that are suspended in a practically water-clear fluid. This is easily observable at the line of contact between the upper surface of the liquid and the side of the containing vessel. When the precipitation is properly made filtration is rapid, requiring usually from two to four hours. It was frequently found convenient to allow filtration to go on overnight. The funnel is then lowered so as to close almost competely the receiving flask. From time to time the filtrates were tested by the addition of more acid or of alkali to make certain that the amounts of acetic acid used were such as to give the maximal precipitation. No difficulty was experienced in correctly judging these amounts.

The number of possible sources of error in this determination is large, and care must be taken to maintain uniform conditions when making these determinations at the beginning and the end of the period.

The average of the two nitrogen determinations was multiplied by $\frac{5}{2}$, as 200 out of 500 c. c. was used, and the result was called (total) water-soluble nitrogen. Obviously this is not absolutely correct, because the volume of the filtrate is 500 c. c. minus the volume of the precipitate. The figures for the increase in water-soluble nitrogen (Table 4) were obtained by subtracting the amounts obtained in June from those obtained in the same way in September.

Results for galactase.—In all of the samples of buttermilk tested water-soluble nitrogen increased very appreciably. The increase was

greatest in the buttermilk from raw cream and least in the buttermilk from cream pasteurized at 93° C. (200° F.). Between 71° and 77° C. (160 and 170° F.) this increase was markedly diminished, indicating that pasteurization between these temperatures strongly inhibited the activity of the galactase. The proteolytic agent, presumably galactase, was not completely destroyed by the pasteurization at any of the temperatures used.

As the pasteurizing temperature was raised the amount of water-soluble nitrogen in the fresh samples was appreciably diminished. All of the filtrates contained protein coagulable by heat in neutral solution, so that the diminished content of water-soluble nitrogen may be due, therefore, to a partial, but not complete, coagulation of milk proteins other than casein.

TABLE 4.—*Influence of pasteurization on the activity of galactase in buttermilk.*

Cream.			Water-soluble nitrogen in 100 c. c. of buttermilk expressed as—								
Sample No.	Pasteurized at—		Cubic centimeters of N/5 nitrogen.			Grams of nitrogen.			Per cent of total nitrogen.		
			June.	Sept.	Increase.	June.	Sept.	Increase.	June.	Sept.	Increase.
	°C.	°F.	c. c.	c. c.	c. c.	Gram.	Gram.	Gram.	Per cent.	Per cent.	Per cent.
46....	Raw.	Raw.	41.8	99.5	57.7	0.117	0.279	0.162	25.3	60.3	35.0
47....	60	140	40.8	90.3	49.5	.114	.253	.139	24.7	54.8	30.1
48....	60	140	35.7	81.3	45.6	.100	.228	.128	21.7	49.3	27.6
58....	66	150	37.5	82.1	44.6	.105	.230	.125	22.7	49.8	27.1
63....	66	150	36.9	80.2	43.3	.103	.225	.122	22.4	48.6	26.2
52....	71	160	33.4	87.8	54.4	.094	.246	.152	20.3	53.3	33.0
51....	71	160	35.7	85.4	49.7	.100	.239	.139	21.7	51.8	30.1
56....	77	170	28.6	59.2	30.6	.080	.166	.086	17.3	35.9	18.6
53....	80	176	33.4	66.6	33.2	.094	.187	.093	20.3	40.4	20.1
54....	82	180	28.0	58.6	30.6	.078	.164	.086	17.0	35.5	18.5
55....	88	190	23.6	42.0	18.4	.066	.118	.052	14.3	25.5	11.2
57....	88	190	27.4	51.5	24.1	.077	.144	.067	16.6	31.2	14.6
59....	93	200	24.0	42.5	18.5	.067	.119	.052	14.6	25.8	11.2

Total nitrogen in 100 c. c. buttermilk=0.462 gram.

TABLE 5.—*Analytic data obtained in Table 4 calculated to average digestion period.*

Cream.			Increase in water-soluble nitrogen in 100 c. c. of buttermilk calculated to 83 days digestion period, expressed as—			Digestion period.	Volume of buttermilk used.	Volume of N/5 acetic acid used for precipitation.	
Sample No.	Pasteurized at—		C. c. of N/5 nitrogen.	Grams of nitrogen.	Per cent of total nitrogen.			June.	Sept.
	°C.	°F.	c. c.	Gram.	Per cent.	Days.	c. c.	c. c.	c. c.
46	Raw.	Raw.	56.3	0.158	34.2	85	50	15	9
47	60	140	48.3	.136	29.4	85	50	13	9
48	60	140	45.1	.126	27.3	84	100	27	20
58	66	150	45.1	.127	27.4	82	200	44	17
63	66	150	44.9	.126	27.2	80	200	41	17
52	71	160	50.2	.140	30.4	90	150	41	14
51	71	160	45.3	.127	27.5	91	150	34	13
56	77	170	31.7	.089	19.3	80	200	44	20
53	80	176	33.2	.093	20.1	83	150	40	21
54	82	180	31.4	.088	19.0	81	200	44	20
55	88	190	18.9	.053	11.4	81	200	44	17
57	88	190	25.0	.070	15.2	80	200	44	28
59	93	200	18.7	.052	11.4	82	200	39	17

According to Babcock, Russell, and Vivian[1] "it is apparent that heating the enzym solutions for 10 minutes at 76° C. suffices to destroy the digestive ferment galactase, and even at 71° C. for the same exposure its action was materially reduced." Hippius[2] found that the proteolytic enzym of cow's milk can withstand an exposure to 65° C. (149° F.) for one-half hour and is not destroyed till near 100° C. (212° F.), at which temperature a short exposure is sufficient. In the light of such results it does not seem remarkable, therefore, that cream pasteurized at 93° C. (200° F.) for about 30 seconds should still contain active galactase. It is of course possible that in pasteurized milk or buttermilk that has been preserved for long periods of time the observed proteolysis is due not alone to undestroyed galactase, but to the hydrolytic action of water as well.

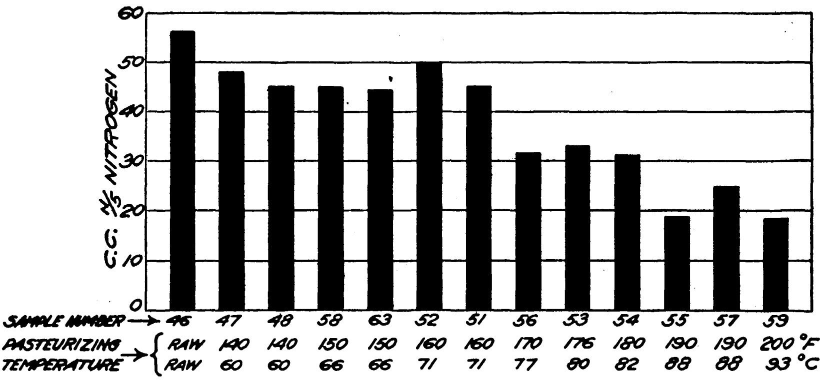

FIG. 24.—Increase in water-soluble nitrogen in 100 c. c. of buttermilk, calculated to 83 days digestion period. (Graphic representation of analytical data in Table 5.)

LIPASE.

Portions of the same sample of raw milk were pasteurized at different temperatures and the activity of the lipase present determined by titrating the amount of acid liberated by it from ethyl butyrate. The apparatus in which the pasteurization was done consisted of a 2½-liter acid bottle containing the milk to be pasteurized. This was inverted over a 5-inch funnel connected by means of rubber and glass tubing with a coil of glass tubing (obtained from two vertical condensers) immersed in a pan containing hot water. From the coil the milk was passed through two ordinary Liebig condensers.

About 3 liters of cold raw milk as received from the dealer was transferred to two large beakers and heated in a hot-air bath to 40°

[1] Babcock, S. M., Russell, H. L., and Vivian, Alfred. Properties of galactase: A digestive ferment of milk. Wisconsin Agricultural Experiment Station, Fifteenth Annual Report, 1898, p. 82.

[2] Hippius, Alexander. Biologisches zur Milchpasteurisierung. Jahrbuch für Kinderheilkunde, Band 61, p. 380. Berlin, 1905.

to 45° C. (104° to 113° F.), requiring 20 to 40 minutes. The milk was transferred to a 2½-liter bottle and inverted over the funnel connected with the pasteurizer. The water in the pan having been previously heated to 90° to 93° C. (194° to 200° F.), the milk was allowed to flow through the glass coil in which the pasteurization took place. The temperature of the milk as it left the coil was indicated by a thermometer placed in it for the purpose. When the milk entering the coil was at 45° C. (113° F.) and the water in the pan at 93° C. (200° F.) the outflowing milk was at 82° C. (180° F.). The burner under the pan was lowered or turned out, according to circumstances, and as the temperature fell the receivers (300 c. c. flasks) at the end of the condenser were changed so as to receive in each one of them the milk that had been pasteurized over a range of temperature not exceeding 2° C. (3.6° F.). In some of the experiments the range was but 1° C. (1.8° F.). The amount of milk received in each flask varied from 150 to 250 c. c. When the pasteurizing temperature was highest the milk as it left the condenser was at 45° C. (113° F.). The receivers were placed in ice and salt for a few minutes to cool the milk still further.

The height of the 2½-liter containing bottle above the coil, etc. (approximately 60 cm.), was so adjusted that the milk passed through the apparatus in about the same time as it does in the "flash" pasteurizer, i. e., 15 to 30 seconds. The pasteurization of 3 liters of milk and its collection in 9 or 10 separate receivers, with the rejection of milk pasteurized at intermediate temperatures, was accomplished without difficulty in 13 to 14 minutes. In general, the pasteurization was conducted as closely as possible under the same conditions as existed in the creamery at the field laboratory. The several series of samples of pasteurized milk were tested for the peroxidase reaction, using tincture of guaiac and dilute hydrogen peroxid solution. The peroxidase reaction disappeared at 77° to 79° C. (170° to 174° F.), indicating that in this respect at least the pasteurization in the two places had been carried out in substantially the same way.

METHOD OF MEASURING THE ACTIVITY OF LIPASE IN MILK.

Into each of two 100 c. c. Erlenmeyer flasks a 50 c. c. portion of the sample was transferred from a measuring cylinder, beginning with the sample that has been pasteurized at the highest temperature. One-half of a cubic centimeter of chloroform and 5 drops of phenolphthalein solution were added to all the flasks. To one of each pair of flasks 0.5 c. c. of ethyl butyrate was added, the other, containing the milk, chloroform, and phenolphthalein mixture, being used as a control. The contents of all the flasks were immediately titrated to a distinct and uniform pink with tenth normal sodium hydroxid, after which the flasks were tightly stoppered with rubber

stoppers. This titration gave the amounts of alkali required to neutralize the acidity of the milk, ethyl butyrate, etc. The flasks were set aside (a thermostat at 26° C. [79° F.] was convenient for this purpose) and rotated about once in 12 hours. At 24-hour intervals the liberated acid was titrated, both in the ethyl butyrate mixtures and in the controls, to a distinct and uniform pink, the color from the previous day's titration having been discharged by the acid liberated. In titrating the controls at the beginning of an experiment approximately 11 c. c. of tenth normal sodium hydrate was required to bring the mixture of milk and chloroform to a reaction faintly alkaline to phenolphthalein. By the following day the pink color had disappeared, and the reaction could again be made alkaline by the addition of approximately 1 c. c. of tenth normal sodium hydroxid. In this way it was ascertained that in 24 hours about 1 c. c. of alkali was in some way consumed, probably by slow combination with some constituent of the mixture. If more than 1 c. c. of alkali was added, the pink color persisted for a longer time; thus, after the addition of an excess of 3 c. c. of alkali, the control remained pink for over 8 days. In mixtures containing milk pasteurized at 74° C. (165° F.) or over (and presumably no lipase) and ethyl butyrate, chloroform, etc., the pink color disappeared more rapidly, which indicated that, in addition to the alkali consumed as described above, a small amount of alkali was used up in hydrolyzing ethyl butyrate.

The figures in the last column of Table 6, headed "Corrected total acid," are the figures in the preceding column minus 6.6 c. c., which represents very closely the amount of alkali consumed in the two above-described ways; i. e., the correction used here represents the amount of alkali consumed in those mixtures in which the lipase was apparently destroyed, and which also contained ethyl butyrate.

Results for lipase.—The figures in Table 6 are typical of three other series giving similar results. It is evident that the lipase was destroyed very near 70° C. (158° F.). It is possible that it was not totally destroyed until a few degrees above this temperature, but the method did not permit the distinction between the very slow hydrolysis of ethyl butyrate by the small amounts of alkali present or by the weakened lipase possibly present in the milk pasteurized above 70° C. (158° F.). An attempt to strengthen the lipase by the addition of an excess of alkali showed this to be inadvisable, because the excess of alkali tended to hydrolyze the ethyl butyrate directly.

The destruction of lipase at somewhat lower temperatures was observed by Terroine,[1] who found that pancreatic juice lost its lipolytic power when heated for 10 minutes at 65° C. (149° F.), and by

[1] Terroine, Emile F. Zur Kenntnis der Fettspaltung durch Pankreassaft. I. Biochemische Zeitschrift, Band 23, p. 424. Berlin, 1910.

Hippius,[1] who found that the lipase in human milk was destroyed when exposed for a short time to a temperature of 64° C. (147° F.)

TABLE 6.—*Influence of pasteurization on the activity of lipase in milk.*

Milk pasteurized at—		N/10 sodium hydroxid required for neutralization of milk, etc.	N/10 acid liberated at end of—					Total acid liberated.	Corrected total acid.
			1 day.	2 days.	3 days.	4 days.	7 days.		
°*C.*	°*F.*	*c. c.*	*c. c.*	*c. c.*	*c. c.*	*c. c.*	*c. c.*	*c. c. N/10.*	*c. c. N/10.*
Raw.	Raw.	12.9	10.2	6.2	4.3	3.0	3.3	27.0	20.4
55	131	12.3	7.8	5.1	3.8	2.8	3.5	23.0	16.4
57	135	12.5	7.7	5.2	4.0	2.5	3.6	23.0	16.4
60	140	12.3	7.6	5.0	3.4	2.5	3.4	21.9	15.3
63	145	12.6	6.3	3.9	3.0	2.1	2.8	18.1	11.5
66	150	12.4	3.7	2.8	2.0	1.7	2.2	12.4	5.8
68	155	12.4	2.5	1.9	1.5	1.3	1.9	9.1	2.5
70	158	12.3	2.2	1.6	1.2	1.2	1.9	8.1	1.5
74	165	12.4	1.7	1.2	1.1	1.2	1.7	6.9	0.3
77	170	12.4	1.9	1.2	1.1	1.1	1.6	6.9	0.3
80	176	13.1	1.6	1.0	1.3	1.0	1.7	6.6	0.0

DISCUSSION OF ENZYM EXPERIMENTS.

In general, the results obtained on the thermal death points of the milk enzyms are in accord with those obtained by other investigators. But close comparisons with their work could not always be made, because the pasteurization process was seldom carried out by two investigators in exactly the same way or for the same length of time. Frequently their descriptions were so imperfect in detail as to make a reproduction of their work, were it desired, practically impossible. For example, one investigator omitted to state how long the pasteurization lasted. In addition, variations in the methods of testing for these enzyms make comparisons still more difficult.

It has been shown that in the pasteurization process catalase and lipase are destroyed at 70° to 71° C. (158° to 160° F.); peroxidase was destroyed at 77° to 79° C. (170° to 175° F.); and galactase, though strongly inactivated between 71° and 77° C. (160° and 170° F.), is not totally destroyed even at 93° C. (200° F.).

It is highly probable that under the pasteurizing conditions prevailing at most creameries that use pasteurized cream for butter making the first two of these enzyms are destroyed. Therefore, as possible factors influencing the keeping qualities of butter made from pasteurized cream, these may be left out of consideration. But on account of their higher thermal death points it is probable that peroxidase is not always destroyed and galactase is rarely, if ever, completely destroyed. Consequently both of these enzyms may be considered possible factors in the deterioration of storage butter.

[1] Hippius, Alexander. Biologisches zur Milchpasteurisierung. Jahrbuch für Kinderheilkunde, Band 61, p. 377. Berlin, 1905.

It has been shown that both of these enzyms are present in buttermilk obtained from cream pasteurized at high temperatures. Researches are now in progress to determine whether the peroxidase is engaged in slowly oxidizing one or more of the constituents of the butter or in assisting in such oxidation. The figures in Table 4 show that the galactase remaining undestroyed in the buttermilk continued to hydrolyze the proteins present. The possible activity of galactase in storage butter is likewise being studied.

It is obvious that a similar study of the action of the other two enzyms would be of interest because of their possible influence on the keeping quality of raw-cream butter.

It must not be inferred that the writers assume a relation between the milk enzyms present in butter and its keeping qualities. The results thus far obtained, however, indicate that the probability of the existence of such a relation is great enough to warrant the studies already undertaken along these lines. The present paper, on the chemical side, is therefore preliminary only.

SCORING THE BUTTER.[1]

The butter made in these experiments was packed in hermetically sealed cans holding about 2 pounds each and stored at −12° C. (10° F.). For scoring, one can was sent to each of three experienced butter judges who worked independently of each other and with no knowledge of the history of the sample. The scores for the first season's work were so inconclusive that they are not included in the tables. In the second season's work a wider range of temperature was used, exceeding the limits of safety at both ends of the range. The first scoring was made after about 40 days in storage, and the second after about 150 days.

The scores are given in Table 7, and presented graphically in figures 25 and 26. The scores of the three judges are not averaged because, while in the main they agree closely, it is believed that on account of varying standards an average will not always indicate the true conditions. The average score at the various temperatures by each scorer is given in Table 8 and shown graphically in figure 26.

The tables show, as would be expected, a marked difference between the unpasteurized and the pasteurized cream butter. They indicate also that pasteurizing temperatures between 60° and 66° C. (140° and 150° F.) leave in the cream some factors causing a deterioration of the butter. Good results were secured from the cream pasteurized at 71° C. (160° F.). At 81° C. (180° F.) some of the samples were marked scorched or cooked.

[1] The authors acknowledge their indebtedness to Mr. P. H. Keiffer, of Gude Bros. Keiffer Co.; Mr. Cromer, of the Fox River Butter Co.; and Mr. John B. Newman, assistant food and dairy commissioner of Illinois, who kindly scored the butter.

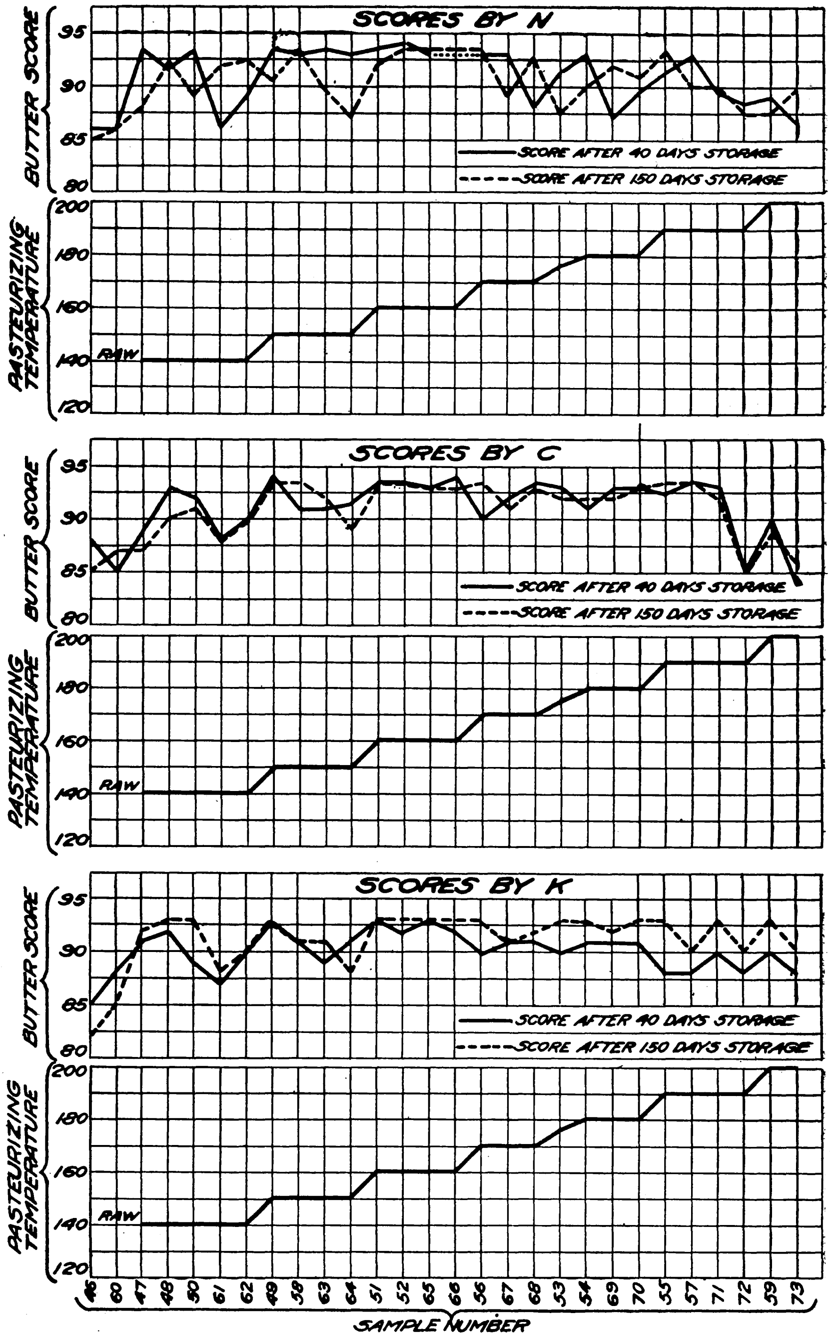

FIG. 25.—Effect of pasteurization temperature of cream on quality of storage butter. (See Table 7.)

At 88° and 93° C. (190° and 200° F.) the quality of the butter was noticeably affected by the high heat, although some of the scorched flavor disappeared on long standing. The temperature at which cream acquires a scorched flavor is no doubt influenced by the amount of fat in the cream, the promptness of the cooling process, the relation of the amount of cream pasteurized to the capacity of the pasteurizer, and possibly other factors. The upper limit of pasteurization therefore can not be determined arbitrarily, but is dependent on varying circumstances. Certainly nothing is gained by exceeding 82° C. (180° F.), and it is probable that there is no advantage in going above 77° C. (170° F.) except the increased certainty of the destruction of the organisms of tuberculosis. The lower limit of efficient pasteurization may be set at 71° C. (160° F.), although under ordinary conditions it will be much safer to use at least 74° C. (165° F.).

It should be remembered, however, that these statements apply only to the conditions under which this work was carried out; that is, the pasteurization, in a continuous machine, of sweet cream for buttermaking. If cream is pasteurized in a vat or other holding device lower temperatures may, undoubtedly, be used.

TABLE 7.—*Effect of pasteurization of cream at various temperatures on quality of storage butter.*

Sample No.	Pasteurizing temperature.		Scored by N.		Scored by C.		Scored by K.	
	° C.	° F.	40 days.	150 days.	40 days.	150 days.	40 days.	150 days.
46	Raw.	Raw.	86	85	88	85	85	82
60	Raw.	Raw.	86	86	85	87	88	85
47	60	140	93½	88	89	87	91	92
48	60	140	91½	92½	93	90	92	93
50	60	140	93½	89	92	91	89	93
61	60	140	86	92	88	88	87	88
62	60	140	89	92½	90	90	90	90
49	66	150	93½	90½	94	93½	93	93
58	66	150	93	93½	91	93½	91	91
63	66	150	93½	89½	91	92	89	91
64	66	150	93	87	91½	89	91	88
51	71	160	93½	92	93½	93½	93	93
52	71	160	94	93½	93½	93½	92	93
65	71	160	93	93½	93	93	93	93
66	71	160	....	93½	93½	93	92	93
56	77	170	93	93½	90	93½	90	93
67	77	170	93	89	92	91	91	91
68	77	170	88	93	93½	93	91	92
53	80	176	91½	87½	93	92	90	93
54	82	180	93	90	91	92	91	93
69	82	180	87	92	93	92	91	92
70	82	180	89½	91	93	93	91	93
55	88	190	91½	93½	92½	93½	88	93
57	88	190	93	90	93½	93½	88	90
71	88	190	89½	90	93	92	90	93
72	88	190	86	87½	85	85	88	90
59	93	200	89	87½	90	89	90	93
73	93	200	86½	90	84	86	88	90

TABLE 8.—*Average butter scores.*

Number of tests.	Pasteurizing temperature.		Scored by N.		Scored by C.		Scored by K.	
	° C.	° F.	40 days.	150 days.	40 days.	150 days.	40 days.	150 days.
2	Raw.	Raw.	86	85½	86½	86	87½	83½
5	60	140	90 7/10	90⅘	90⅕	89⅕	89⅘	91⅕
4	66	150	93¼	90⅛	91 1/10	92	91	90¾
4	71	160	93⅓	93⅛	93 3/10	93¼	92½	93
3	77	170	91⅓	91⅚	91⅘	92½	90⅔	92
1	80	176	91½	87½	93	92	90	93
3	82	180	89⅝	91	92⅓	92⅓	91	92⅔
4	88	190	90	90¼	91	91	88½	91½
2	93	200	87¾	88¾	87	87½	89	91½

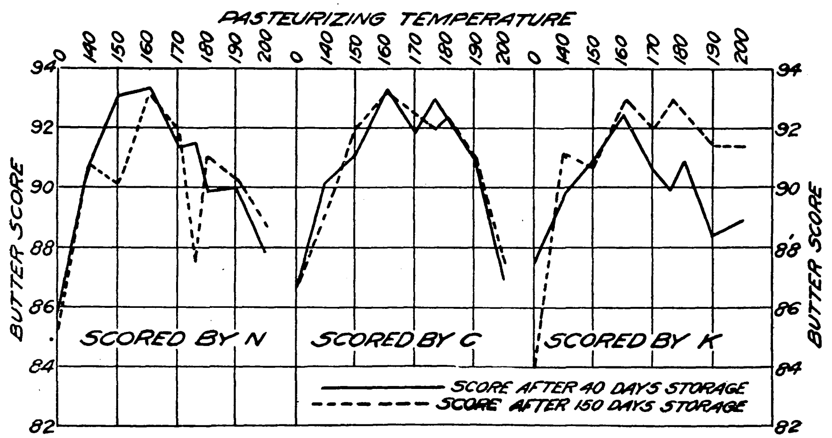

FIG. 26.—Average scores of butter, showing effect of pasteurization of cream at various temperatures.

SUMMARY.

The proper temperature for the pasteurization in a continuous machine of sweet cream for butter making was determined by observing (1) the temperature at which the greater part of the bacteria were destroyed, (2) the temperature at which the various enzyms of the milk were inactivated, and (3) the relative keeping quality of butter made from cream pasteurized at different temperatures.

The uniform destruction of the vegetative bacteria is uncertain at temperatures below 74° C. (165° F.).

Peroxidase was destroyed at 77° C. (170° F.), catalase and lipase at 70° C. (158° F.). Galactase was much weakened by temperatures between 71° C. (160° F.) and 77° C. (170° F.), but was not destroyed at 93° C. (200° F.), the highest temperature employed.

Examination of the butter after storage indicated that pasteurization at 66° C. (150° F.) or lower left in the cream some factor causing a deterioration of the butter. This was not evident in the butter from cream pasteurized at the next higher temperature, 71° C. (160° F.), or higher. At 82° C. (180° F.) the flavor of the butter was affected by the heat. This action, however, may be controlled to some extent by the skill of the butter maker.

For the continuous pasteurization of sweet cream for butter making a temperature not lower than 74° C. (165° F.) nor higher than 80° C. (175° F.) is recommended.

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